

- 5 15. The vaccine of claim 11, wherein the bacterium is a laboratory strain of E. coli.
- 6 16. The vaccine of claim 11, wherein the bacterium is dead or non-viable.
- 7 17. The vaccine of claim 11, wherein the bacterium comprises the cytolysin.
- 8 18. The vaccine of claim 11, wherein the agent is synthesized by the bacterium.
- 9 19. The vaccine of claim 11, wherein the bacterium is engineered to deliver to antigen-presenting cells antigenic polypeptides which are presented in association with MHC proteins.
- 10 20. The vaccine of claim 11, wherein the bacterium is nonreplicative and nonintegrative into the host cell genome.
- 11 21. The vaccine of claim 11, wherein the bacterium is a dead or nonviable laboratory strain of E. coli.
- 12 22. The vaccine of claim 11, wherein the bacterium is a laboratory strain of E. coli and the bacterium comprises the cytolysin.
- 13 23. The vaccine of claim 11, wherein the bacterium is a dead or nonviable laboratory strain of E. coli and the bacterium comprises the cytolysin.
- 14 24. The vaccine of claim 11, wherein the bacterium is a dead or nonviable laboratory strain of E. coli and the bacterium comprises the cytolysin and the cytolysin is listeriolysin.
- 15 25. The vaccine of claim 11, wherein the bacterium is a laboratory strain of E. coli engineered to deliver to antigen-presenting cells antigenic polypeptides which are presented in association with MHC proteins.

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26. A pharmaceutical composition comprising a nonvirulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding a different foreign therapeutic agent.

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17 *27.* The composition of claim *26*, wherein the cytolysin is absent a functional signal sequence.

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18 *28.* The composition of claim *26*, wherein the cytolysin is listeriolysin.

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19 *29.* The composition of claim *26*, wherein the bacterium further comprises a third gene encoding an invasin and a fourth gene encoding an autolysin.

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20 *30.* The composition of claim *26*, wherein the bacterium is a laboratory strain of E. coli.

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21 *31.* The composition of claim *26*; wherein the bacterium is dead or non-viable.

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22 *32.* The composition of claim *26*, wherein the bacterium comprises the cytolysin.

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23 *33.* The composition of claim *26*, wherein the agent is synthesized by the bacterium.

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24 *34.* The composition of claim *26*, wherein the bacterium is nonreplicative and nonintegrative into the host cell genome.

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25 *35.* The composition of claim *26*, wherein the bacterium is a dead or nonviable laboratory strain of E. coli.

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26 *36.* The composition of claim *26*, wherein the bacterium is a laboratory strain of E. coli and the bacterium comprises the cytolysin.



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27 31. The composition of claim 26, wherein the bacterium is a dead or nonviable laboratory strain of E. coli and the bacterium comprises the cytolysin.

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28 38. The composition of claim 26, wherein the bacterium is a dead or nonviable laboratory strain of E. coli and the bacterium comprises the cytolysin and the cytolysin is listeriolysin.

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29 39. The composition of claim 26, wherein the agent is selected from an antibiotic, insecticide, fungicide, anti-viral agent, anti-protozoan agent, enzyme, anti-cancer agent, antibody, anti-inflammatory peptide, and transcription factor.

SUS 31
30 40. A method of generating an immune response comprising the step of introducing a foreign antigenic agent into a eukaryotic cell comprising the step of contacting the cell with the bacterium of claim 1 under conditions whereby the agent enters the cell.

31 30 41. The method of claim 40, wherein the bacterium is endocytosed into a vacuole of the cell, the bacterium undergoes lysis and the cytolysin mediates transfer of the agent from the vacuole to the cytosol of the cell.

32 30 42. The method of claim 40, wherein the bacterium is dead or non-viable.

33 30 43. The method of claim 40, wherein the bacterium comprises the cytolysin.

34 30 44. The method of claim 40, wherein the agent is synthesized by the bacterium.

35 30 45. The method of claim 40, wherein the bacterium is engineered to deliver to antigen-presenting cells antigenic polypeptides which are presented in association with MHC proteins.

36 30 46. The method of claim 40, wherein the bacterium is nonreplicative and nonintegrative into the host cell genome.

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37. The method of claim ⁴⁰, wherein the bacterium is a dead or nonviable laboratory strain of E. coli.

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38. The method of claim ⁴⁰, wherein the bacterium is a laboratory strain of E. coli and the bacterium comprises the cytolysin.

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39. The method of claim ⁴⁰, wherein the bacterium is a dead or nonviable laboratory strain of E. coli and the bacterium comprises the cytolysin.

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40. The method of claim ⁴⁰, wherein the bacterium is a dead or nonviable laboratory strain of E. coli and the bacterium comprises the cytolysin and the cytolysin is listeriolysin.

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41. The method of claim ⁴⁰, wherein the bacterium is a laboratory strain of E. coli engineered to deliver to antigen-presenting cells antigenic polypeptides which are presented in association with MHC proteins.

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42. The method of claim ⁴⁰, wherein there is no growth or metabolism of the bacterium in the eukaryotic cell.

Sub B2
53. A method for treating a disease comprising introducing a foreign therapeutic agent into a eukaryotic cell comprising contacting the cell with the bacterium of claim 1 under conditions whereby the agent enters the cell.

54. The method of claim 53, wherein the bacterium is endocytosed into a vacuole of the cell, the bacterium undergoes lysis and the cytolysin mediates transfer of the agent from the vacuole to the cytosol of the cell.

55. The method of claim 53, wherein the bacterium is dead or non-viable.

56. The method of claim 53, wherein the bacterium comprises the cytolysin.